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Synthesis and Collateral Dilator Activity of Nitroxyalkylamides Having Direct or Latent Sulfhydryl Moieties

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Abstract—To develop an orally active, long-acting nitrate that does not induce tolerance, nitroxyalkyl compounds were prepared and their activities evaluated by the use of carotid collaterals in anesthetized dogs. A compound having a favorable pharmacological profile, that is, long-lasting collateral vasodilatation and little hypotension, and lack of nitrate tolerance, was chosen for further evaluation.

Introduction

Organic nitrates[1] have been widely used as antianginal drugs. Since nitroglycerin (**1**) and isosorbide dinitrate[2] undergo a hepatic first-pass effect and have a short duration of action, they are administered by the sublingual route for the treatment of angina pectoris or by the transdermal route for prophylaxis. An orally active sustained-release preparation of isosorbide 5-mononitrate is also widely used. Nitrates have the disadvantage of causing adverse reactions such as dizziness, headache or tachycardia, which are induced by their potent hypotensive actions. Another disadvantage of these drugs is the development of nitrate tolerance following their continuous use, which is regarded to be the most serious clinical limitation shared by all of the nitrates under clinical use. We, therefore, set a goal of developing a new nitrate compound having the following pharmacological properties: (1) a long-lasting collateral vasodilator effect, (2) minimal hypotensive effect and tachycardia, (3) no hepatic first-pass effect and (4) lack of tolerance development. Nitrate drugs are mostly prodrugs which release nitric oxide (NO),[3] the active intermediate, in vascular smooth muscle cells. Recently, mitochondrial aldehyde dehydrogenase (mtALDH) has been identified by Stamlar et al.[4] as an enzyme involved in the bio-activation of nitroglycerin. The activity of this enzyme has been shown to be dependent on the presence of sulfhydryl as a reductant. They have also demonstrated that desensitization of mtALDH due to a continuous exposure to nitroglycerin might account for tolerance development.

We designed and synthesized nitrate compounds that simultaneously release NO and thiols in the cell. In the present study, focusing on the cysteinyl group of glutathione, cysteine derivatives (**5**) and 2-oxothiazolidine derivatives (**7**)[5] were synthesized. 2-Oxothiazolidine-4-carboxylic acid is a latent cysteine since it is converted in vivo into cysteine by the action of an enzyme, 5-oxoprolinase.[6] The cysteine derivatives and 2-oxothiazolidine derivatives are expected to prevent the development of tolerance by generating sulfhydryl group in vivo.

In the present study, the pharmacological activity of cysteine derivatives and 2-oxothiazolidine derivatives was evaluated for their vasorelaxant effect on carotid collateral vessels in anesthetized dogs.

Synthesis

The synthetic method of compounds **5a–j**, **7a–t** having a nitroxyalkylamide group is shown in Scheme 1.

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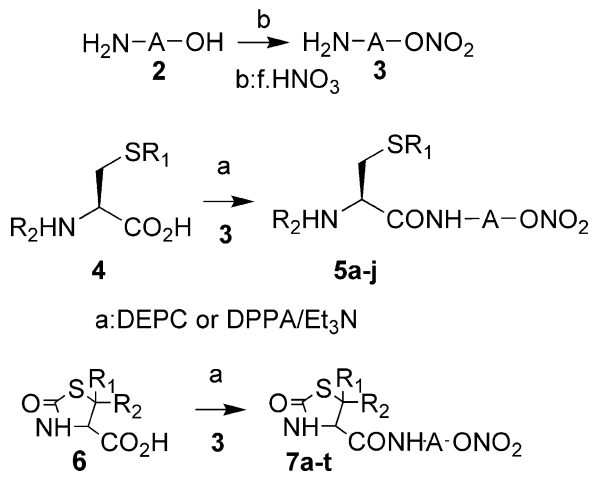


Scheme 1.

Compounds **5a–j** and **7a–t** were synthesized from the corresponding cysteine derivatives (**4**) and 2-oxothiazolidine-4-carboxylic acid derivatives (**6**) with nitroxyalkylamine derivatives (**3**) by a condensation reaction with DEPC or DPPA in the presence of triethylamine (Et_3N).

Nitroxyalkylamine derivatives (**3**) were prepared by adding aminoalcohol derivatives (**2**) to fuming nitric acid.

The synthesized compounds are shown in Tables 1 and 2.

Screening Procedure

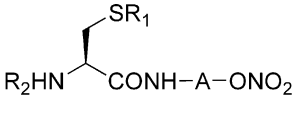
Male adult beagle dogs were used in this experiment. Animals were anesthetized with sodium pentobarbital (40 mg/kg iv) and artificially ventilated. The femoral arterial blood pressure and heart rate were monitored throughout the experiment.

The characteristics of collateral vessels in the carotid vascular bed were investigated in detail in the anesthetized dogs by Iriuchijima and Koike.[7,8] Based on their results, we have developed a new method to evaluate the vasodilator effect of a test compound on collateral arteries. The details of the method will soon be published elsewhere, but here is a brief description.

A polyethylene catheter (2F) was inserted into one of the branches of the left thyrocervical artery for recording the left carotid arterial pressure (CAP). The common left carotid artery was occluded for 1 min, proximal to the insertion site of the polyethylene catheter, and CAP was measured immediately before and after occlusion. The decrease in CAP (ΔP) was measured during occlusion. Prior to the administration of a test substance, the carotid occlusion was repeated every 15 min until the same response was obtained at least for three successive measurements. The test substance was intravenously administered at a dose of 0.1 or 0.3 mg/kg, and the left carotid artery was occluded for 1 min at 5, 15, 30, 45 and 60 min after the administration. At each time point, CAP immediately before occlusion (P') and the decrease in CAP after occlusion (DP') were measured. The vasodilator activity of the compound in the collateral vessels (CI = collateral index) was calculated at each measurement time point according to the following equation. $CI = 100-(\Delta P'/P')\times 100/(\Delta P/P)$.

Thereafter, the AUC (CI_{AUC}) over a period of 60 min was also calculated. As a reference substance, nitroglycerin was used. It was administered intravenously at a dose of 0.03 mg/kg, and the CI_{AUC} was calculated. From the values obtained above, the ratio of the CI_{AUC} value of each test substance to that of nitroglycerin (NTG ratio) was calculated.

Table 1. The synthesized compounds (**5a–j**)

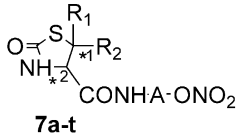


5a-j

Compd	R1	R2	A	mp
5a	H	Ac	$(CH_2)_2$	103–104
5b	H	Nic	$(CH_2)_2$	99–100
5c	Ac	Ac	$(CH_2)_2$	95–97
5d	iByr	iByr	$(CH_2)_2$	64–66
5e	Piv	Ac	$(CH_2)_2$	101–103
5f	Boz	Ac	$(CH_2)_2$	137–138
5g	Nic	Ac	$(CH_2)_2$	110–112
5h	Etc	Ac	$(CH_2)_2$	119–120
5i	jBuc	Ac	$(CH_2)_2$	92–93
5j	Boc	Ac	$(CH_2)_2$	96–97

Ac: acetyl; iByr: isobutyryl, Piv: pivaloyl, Boz: benzoyl, Nic: nicotinoyl, Etc: ethoxycarbonyl, iBuc: isobutyloxy-carbonyl, Boc: t-buoxycarbonyl.

Table 2. The synthesized compounds (**7a–t**)



7a-t

Compd	R1	R2	*1	*2	A	mp
7a	H	H	—	*R*	$(CH_2)_2$	133–134
7b	H	H	—	*S*	$(CH_2)_2$	129–130
7c	H	H	—	*R*	$(CH_2)_3$	83–85
7d	H	H	—	*R*	$(CH_2)_4$	68–70
7e	H	H	—	*R*	$CH(CH_3)CH_2$	125
7f	H	H	—	*R*	$CH(CH_3)CH_2$	102–103
7g	H	H	—	*S*	$CH(CH_3)CH_2$	102–103
7h	H	H	—	*S*	$CH(CH_3)CH_2$	119
7i	H	H	—	*R*	$CH_2CH(CH_3)$	oil
7j	H	H	—	*R*	$CH(Et)CH_2$	106–107
7k	H	H	—	*R*	$CH(Pr)CH_2$	99–100
7l	H	H	—	*R*	$CH(Bu)CH_2$	110–112
7m	Me	H	*R*	*R*	$(CH_2)_2$	oil
7n	Me	Me	—	*R*	$(CH_2)_2$	98–100
7o	Ph	H	*RS*	*RS*	$(CH_2)_2$	138–140
7p	Bz	H	*RS*	*RS*	$(CH_2)_2$	123
7q	2-Furyl	H	*RS*	*RS*	$(CH_2)_2$	117–118
7r	2-Thienyl	H	*RS*	*RS*	$(CH_2)_2$	120
7s	1-Naphtyl	H	*RS*	*RS*	$(CH_2)_2$	151
7t	3-Pyridyl	H	*RS*	*RS*	$(CH_2)_2$	189

*1 and *2 indicate the stereochemistry of asymmetric carbon atoms. The asymmetric center of the A moieties of **7e**, **7g** and **7j–l** are *S*, that of **7f** and **7h** are *R*, and that of **7i** are *R*, *S*. Me: methyl, Ph: phenyl, Bz: benzyl.

The vasodilator activity of each test substance in the collateral vessels was evaluated based on (1) the maximum dilatation effect (max *CI*), (2) CI value at 60 min after administration, (3) ratio of AUC of each test substance to that of nitroglycerin and (4) the maximum decrease in femoral arterial blood pressure (maximum hypotension, as shown in Tables 3 and 4).

Dilatation Activity on Carotid Collaterals

NTG (**1**) was administered intravenously at a dose of 0.03 mg/kg. After 5 min, the dilatation of collateral vessel reached a maximum ($CI_{max} = 33$) and then, diminished rapidly ($CI_{60} = 4$ in Table 3, and Fig. 1).

The femoral arterial blood pressure fell markedly immediately after administration of NTG. This was also accompanied by marked tachycardia.

The collateral dilator and hypotensive actions of cysteine derivatives (**5a–j**) are summarized in Table 3.

Table 3. Collateral Index of nitroxyalkyl compounds (**5a–j**) in anesthetized dog

Compd	Dose (mg/kg, iv)	Max *CI*	*CI* (at 60 min)	NG ratio	Max hypotension (%)
1	0.03	33	4	1.0	−55
5a	0.1	39	9	1.9	−52
5b	0.1	2	0	0.0	−3
5c	0.1	52	6	2.0	−44
5d	0.1	25	9	1.5	−14
5e	0.1	45	8	2.5	−23
5f	0.1	49	5	2.1	−28
5g	0.1	20	9	1.1	−24
5h	0.1	39	16	2.4	−18
5i	0.1	25	6	1.3	−12
5j	0.1	21	11	0.8	−8

Table 4. Collateral Index of nitroxyalkyl compounds (**7a–t**) in anesthetized dog

Compd	Dose (mg/kg, iv)	Max *CI*	*CI* (at 60 min)	NG ratio	Max hypotension (%)
1	0.03	33	4	1.0	−55
7a	0.1	23	16	1.9	−8
7b	0.3	14	11	0.8	−6
7c	0.3	4	6	0.1	0
7d	0.1	2	0	0.0	0
7e	0.3	16	15	1.1	−5
7f	0.3	12	12	0.9	−4
7g	0.3	5	3	0.3	−1
7h	0.3	6	5	0.4	−3
7i	0.1	0	0	0.0	0
7j	0.3	7	7	0.5	−1
7k	0.3	18	17	1.3	−7
7l	0.3	21	17	1.6	−4
7m	0.1	9	4	0.5	−2
7n	0.3	9	8	0.6	−2
7o	0.3	18	9	1.0	−6
7p	0.1	12	7	1.0	−3
7q	0.3	6	3	0.4	−3
7r	0.1	8	3	0.5	0
7s	0.1	9	9	0.5	−2
7t	0.1	2	0	0.1	−4

Among 10 cysteine derivatives examined, 8 showed collateral dilator action that was as potent as NTG, but the duration of the action was relatively short. In addition, these cysteine derivatives induced marked hypotension. Thus, the pharmacological profile of each of the cysteine derivatives was very similar to that of NTG and the collateral dilator action of the cysteine derivatives seemed to be inseparable from their hypotensive action.

The effects of 2-oxothiazolidine derivatives (**7a–t**) on collateral vessels and systemic blood pressure are shown in Table 4, when the derivative compounds were given at doses of 0.1 or 0.3 mg/kg, iv The collateral dilator activity of most 2-oxothiazolidine derivatives was less than that of NTG or cysteine derivatives at the peak response. However, their action was much longer than cysteine derivatives, such as compounds **7a**, **7e**, **7f**, **7k** and **7l**. The hypotensive action of these 5 compounds was negligible.

Of compounds **7a–t**, compounds in which the R_1 and R_2 were hydrogen atoms exhibited the highest activity and introduction of alkyl, phenyl or heterocyclic groups at R_1 diminished the activity. Regarding the alkylene group shown as moiety A, compound **7a** having a straight chain alkylene group showed the highest activity, and the activity of compounds **7c–d** with a longer straight chain was low, compared with that of **7a**. Introduction of branched alkyl groups gave rise to an asymmetric center and decreased the activity, as seen with **7e–j**. However, a much more bulky alkyl group seemed to increase the activity.

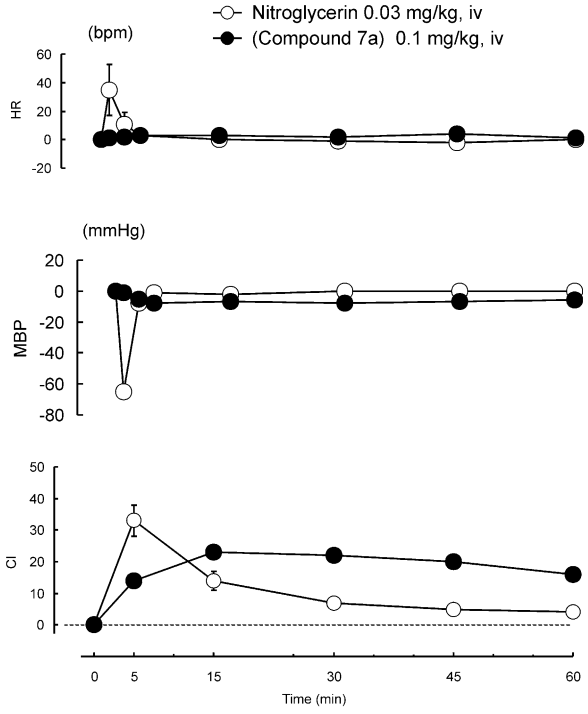


Figure 1. Effects of nitroglycerin and compound **7a** on hemodynamics and carotid collateral vessels in anesthetized dogs. HR and MBP are expressed as absolute changes from baseline values. All data are means ± standard errors of 7 experiments. Abbreviations, HR: heart rate, MBP: mean blood pressure, CI: collateral index.

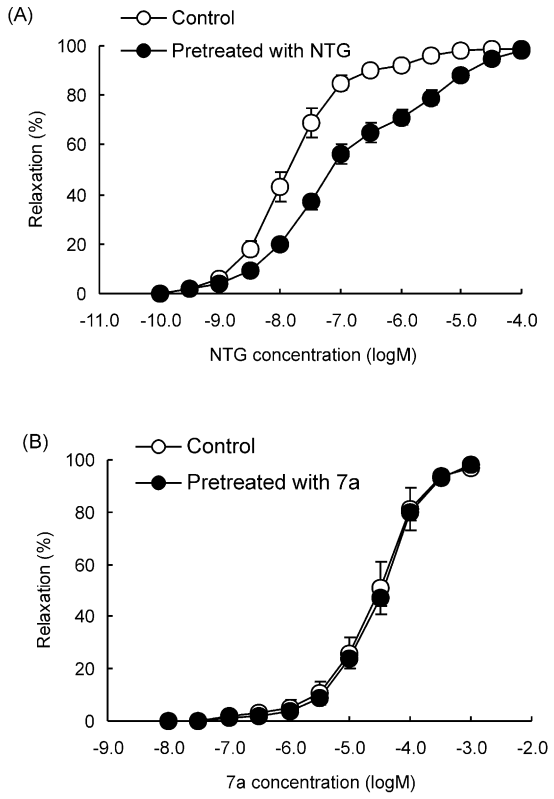


Figure 2. Vasorelaxant effects of nitroglycerin (NTG: A) or compound **7a** (**7a**: B) in isolated rat aortic ring segments pretreated with the vehicle (control) and nitroglycerin or compound **7a**, respectively. The aortic ring segments were pre-incubated for 60 min with the vehicle and NTG or compound **7a** at a concentration of 10^{-4} M or 10^{-3} M, respectively, washed repeatedly with drug-free fresh buffer for 60 min, and pre-contracted with phenylephrine at a concentration of 3×10^{-7} M, followed by cumulative administration of NTG or compound **7a**, respectively. Data are means±SE of 3–5 experiments.

The stereoconfiguration at 4-position of the thiazolidine ring of compounds **7a–t** affected the activity; *R* stereoisomers derived from natural amino acids generally showed more potent activity than *S* isomers.

The vasodilator effects of compound **7a** on collateral vessels developed slowly and reached a maximum ($CI_{max}=23$) at 15 min after the administration, but the dilator action lasted until the end of the experiment ($CI_{60}=16$). In contrast to NTG, compound **7a** produced little hypotension and reflex tachycardia. Thus, compound **7a** had a potent and long-lasting dilator activity of collateral vessels and a favorable hemodynamic effect.

Furthermore, we examined whether 2-oxothiazolidine derivatives developed nitrate tolerance in isolated rat aorta or not. Tolerance was induced by pre-incubation for 60 min with a high concentration of NTG (10^{-4} M) or compound **7a** (10^{-3} M), followed by a washout period of 60 min. Pre-incubation with each drug did not change the degree of contraction induced by phenylephrine (data not shown). As shown in Figure 2A, the vasorelaxant effects of NTG in aortic strips pretreated with 10^{-4} M NTG were markedly attenuated as compared with those pretreated with the vehicle, suggesting the development of tolerance. On the contrary, the vasorelaxant activity of compound **7a** was not affected by pre-incubation, not even at the highest concentration of 10^{-3} M, as shown in Figure 2B.

In summary, we synthesized organic nitrates with direct (cysteine derivatives) and latent (2-oxothiazolidine derivatives) sulfhydryl moieties. Some nitrates with latent sulfhydryls seemed to have favorable pharmacological properties as antianginal drug: (1) a long-lasting collateral vasodilator effect, and (2) minimal hypotensive effect and tachycardia. In particular, among these derivatives, compound **7a** induced no nitrate tolerance.

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